AUTOMATED MICROFLUIDIC SYSTEM FOR RAPID GENERATION OF LIBRARIES OF NANOLITER DROPLETS

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ABSTRACT

We demonstrate a system for rapid generation of libraries of nL droplets from sequences of μL droplets of arbitrary compositions. This system forms a useful link between the automated systems for high-throughput generation[3] of reaction conditions and those for high-throughput screening of random libraries[1,2].

KEYWORDS: microdroplets, splitting, flow-focusing, droplet libraries

INTRODUCTION

Droplet microfluidics challenges the classical robotic stations in high-throughput screening applications, offering smaller reaction volumes and up to thousands of repetitions while reducing the cost and time of the protocols. Still, several challenges remain to be tackled. One hurdle is in the lack of facile methods for rapid and automated generation of libraries of nL droplets containing a range of concentrations and combinations of the chemistry to be tested in a high-throughput screen. The reported systems [1,2] offer only efficient screening of random libraries but cannot generate e.g. different concentrations of tested compounds or factors (e.g. affecting mutation rate) without off-chip manipulations.

EXPERIMENTAL

We milled the chip (using CNC mill Ergwind MSG4025, Poland) into a slab of polycarbonate (Makroclear, Bayer, Germany) and assembled by 30 min thermal bonding at 130°C in press with a pressure 0.4 MPa. The dimensions of channels were varied from 100 μm to 800 μm. Distilled water dyed by 12,5% solution red ink (Waterman, France) served as the droplet phase. We used two types of continuous liquid: oil that transports mother droplets was pure hexadecane, whilst crossflow liquid that splits mother droplet into droplet library was 2 % w/w solution of Span80 in hexadecane. We used droplet on demand technology, developed in our group [3]. We used pressurized reservoirs of liquids interfaced with modified (as described previously by Churski et al. [3]) electromagnetic valve (V165, Sirai, Italy) Steel capillaries (I.D. 0.21 mm, Mifam, Poland) of high hydraulic resistance (2.95·1012 kg m^{-4} s^{-1}) and of length equal to 200 cm served as connections between valve and polycarbonate chip and they were used in order to ensure stable formation of droplets. For connections between the capillaries the needles we used elastic Tygon tubing (inner diameter 0.25 mm, outer diameter 2 mm, Ismatec, Switzerland). Every channel began with round perpendicular hole fitted for 21 gauge needle of outer diameter of 0.82 mm.

All of the inlets to the system are controlled with electromagnetic valves. This allows for complete synchronization of all the processes, including formation of parent droplets, rapid (> hundreds of Hz) splitting of these droplets in the flow focusing module and introduction of gaseous spacers between the families of nL droplets.

RESULTS AND DISCUSSION

We demonstrate an integrated system for rapid generation of libraries of droplets. Our device is fabricated in polycarbonate and uses external valves [3] to first generate droplets on-demand from three sources of solutions, merge these droplets into ~ 1 μL mixtures (Fig.1). These parental plugs are subsequently injected either on the same chip, or onto a second chip via a tubing connector where they are fragmented in a flow-focusing module into thousands of daughter monodisperse droplets of subnanoliter volumes.

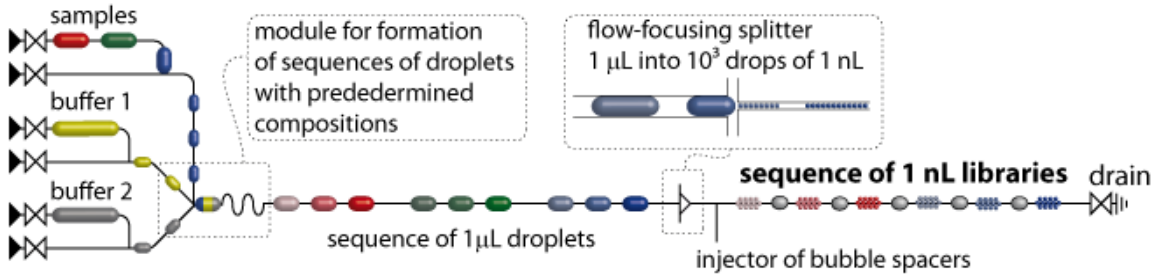

Figure 1: : Schematics of the microfluidic system for rapid generation of droplet libraries

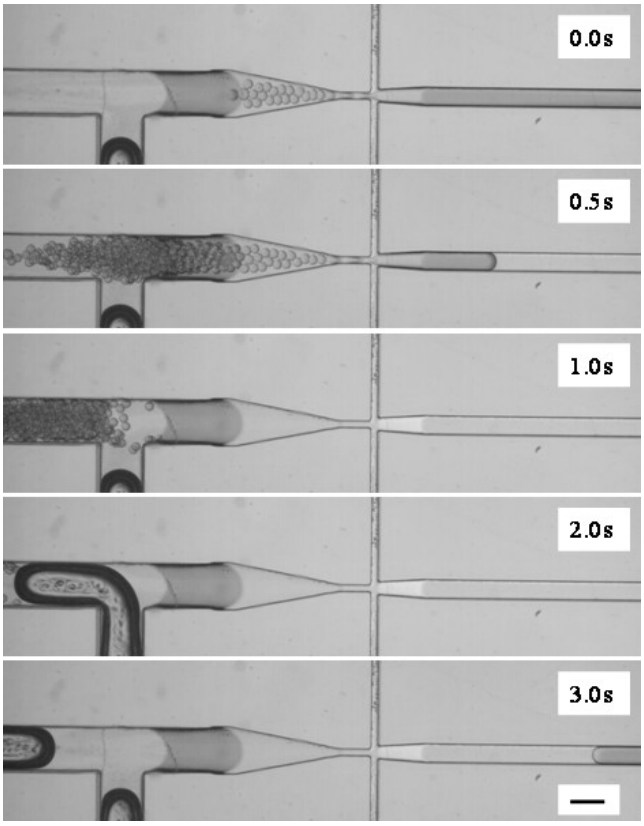

Figure 2. Micrographs illustrating generation of an exemplary library. The parent droplet with volume 1100 nl was divided into 1300 daughter droplets (volume ~0.84 nl). Volume can be regulated by changing flow rate of crossflow stream of oil. Each library is generated with frequency 0.32 Hz. Each element of the library is generated with frequency ~700 Hz. Red ink (Waterman) was used as aqueous phase. Scale bar is 500 μm.

We found that in order to minimize the dispersion of the volumes of these daughter droplets it is necessary to gradually decrease the width of the microfluidic channel upstream of the FF module and that the whole parent droplet must be squeezed into the section of a narrow channel before it enters the orifice of the FF splitter. This procedure omits the problems associated with the changes of the curvature of the parent droplet and thus diminishes influence of Laplacian pressure on the process of formation of daughter droplets (Fig. 3). Another crucial step that ensured production of monodisperse daughter droplets was removal of surfactant from continuous phase of mother droplet stream.

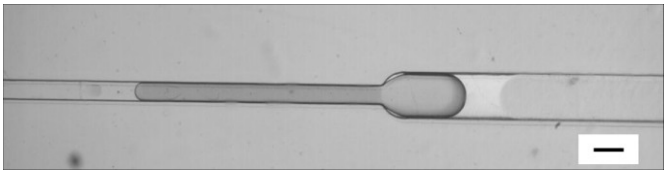

Figure 3. Process of elongation of droplet – the wider channel is 800x800 microns and the smaller channel is 300x300 microns. Scale bar is 500 μm

All of the inlets to the system are controlled with electromagnetic valves. This allows for complete synchronization of all the processes, including formation of parent droplets, rapid (> hundreds of Hz) splitting of these droplets in the flow focusing module and introduction of gaseous spacers between the families of nL droplets. Libraries of daughter droplets are can be transported in tubing or within cylindrical channels (Fig.4). Daughter droplets are highly monodisperse with CV~1,5% (Fig.5).

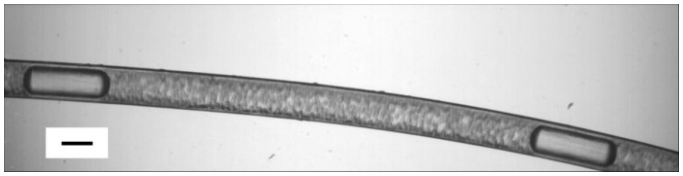

Figure 4. Polyethylene tubing containing droplet libraries separated with air bubbles. Scale bar is 1 mm

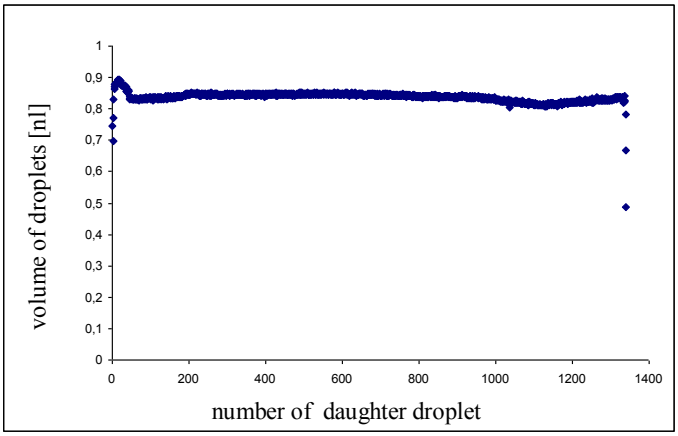

Figure 5. Graph of the distribution of droplet volumes. Generally droplets are highly monodisperse, except a few first and last ones (no more than five daughter droplets differ significant in size). Droplets were measured by software ImageJ.

CONCLUSION

The system that we describe shows the complementation of the automated systems[3] for generation of large (i.e. ~ 1 μL) plugs of arbitrary composition with the methods[1,2] for high-throughput screening of small (i.e. ~ nL) droplet libraries. Our device can serve as a crucial improvement in the single cell or single molecule microfluidic screening assays and droplet digital PCR systems. Additionally this systems can be used in environmental microbiology, ecology studies of bacteria communities (cross-feeding and prey-predator systems, co-cultures, enrichment and/or isolation of specific strains and mutants), stem cell research (determination of artificial stem cell niche, screening on growth factors) and biochemistry (screening on single enzyme molecules, directed evolution).

ACKNOWLEDGEMENTS

Project operated within the Foundation for Polish Science (FPS) Team Program (2008-1/1) co-financed by the EU European Regional Development Fund.

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